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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/055,711
Filing Date: January 22, 2002
Appellant(s): REBAR ET AL.

Dahna S. Pasternak
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 7/7/2009 appealing from the Office action mailed 4/27/2009.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

Appellants note that an Appeal Brief was filed on November 27, 2006 in U.S. Serial No. 10/470,180; however this appeal is not considered relevant to the instant case on appeal. The facts at hand in the instant case are not the same as those of U.S. Serial No. 10/470,180. The claims of each application are directed to different inventions.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

7,151,201 B2	BARBAS, III et al.	12-2006
7,329,728 B1	BARBAS, III et al.	2-2008
5,789,538	REBAR et al.	8-1998
WO 96/06166	GILMAN et al.	2-1996
WO 00/23464	BARBAS et al.	4-2000

Filippova et al. "An Exceptionally Conserved Transcriptional Repressor, CTCF, Employs Different Combinations of Zinc Gingers to Bind Diverged Promoter Sequences of Avian and Mammalian c-myc Oncogenes." Molecular and Cellular Biology, Vol. 16, No. 6 (June 1996), pp. 2802-2813.

Guyer et al. "Activation of Latent Transgenes in Arabidopsis Using a Hybrid Transcription Factor." Genetics, Vol. 149 (1998), pp. 633-639.

Desjarlais et al. "Use of a Zinc-Finger Consensus Sequence Framework and Specificity Rules to Design Specific DNA Binding Proteins." Proceedings of the National Academy of Sciences, USA, Vol. 90, No. 6 (March 1993), pp. 2256-2260.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 25-28, 30-32, 36-37, 39-41, 53-57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Barbas, III et al (US Patent No. 7,151,201 B2, cited in a prior action; see the entire reference) in view of Filippova et al (Molecular and Cellular Biology, Vol. 16, No. 6, pages 2802-2813, June 1996; see the entire reference).

Barbas, III et al teach nucleic acid molecules encoding zinc finger proteins that bind to a target nucleotide sequence of 3, 6, 9, 12, 15 or 18 nucleotides, where the zinc finger protein binds the target nucleotide sequence of the formula $(GNN)_n$, where N is any one of A, T, C or G and n is an integer from 1 to 6 (e.g., column 3, lines 13-43; column 18, lines 48-64; column 19,

lines 53-57; Table 2). The region of the zinc finger protein that mediates the specific binding spans positions -1 to +6 of the alpha helix and, thus, is a recognition helix of seven amino acids in length (e.g., column 21, lines 34-39; boxed sequences in Figure 6). Barbas, III et al teach that any naturally occurring zinc finger protein can be used as a framework (or backbone) to derive a non-naturally occurring zinc finger with DNA binding specificity determined by alterations in the alpha helix using known design rules (e.g., column 10, lines 55-67; column 11, lines 14-35; column 19, lines 28-34 and 53-57; column 21, lines 8-39; column 22, line 51 to column 25, line 9). Barbas, III et al teach that the target nucleotide sequence can be present in a plant cell and can be a promoter sequence (e.g., column 3, lines 23-50). Further, Barbas III et al teach that the target nucleotide sequence can be endogenous or exogenous to the target gene (e.g., column 3, lines 23-50). Barbas, III et al teach that the encoded zinc finger protein also includes an activation domain of a regulatory protein, such as a C1 activator domain of maize, in order to activate expression of the target gene operably linked to the target nucleotide sequence (e.g., column 4, lines 42-48; column 25, lines 10-46). Barbas, III et al teach expression vectors comprising the polynucleotide sequences encoding the zinc finger proteins, and plant host cells comprising the vectors (e.g., column 32, lines 10-36). Barbas, III et al teach the suspension of the polynucleotides in a pharmaceutically acceptable excipient that is an electroporation buffer of 0.3 M mannitol, 5 mM MES, 70 mM KCl, pH 5.8 (e.g., column 55, lines 35-67).

Barbas, III et al do not teach the isolated polynucleotide, where the polynucleotide encodes a non-canonical zinc finger component comprising a beta turn comprising two amino-terminal zinc coordinating cysteine residues separated by two amino acids and an alpha helix comprising one carboxy-terminal zinc coordinating histidine residue and one carboxy-terminal

cysteine residue, where the carboxy-terminal histidine residue is amino terminal to the carboxy-terminal cysteine residue and the histidine and cysteine residues are separated by three amino acids.

Filippova et al teach nucleic acid molecules encoding the 11 zinc fingers of CTCF protein (e.g., page 2803, Isolation of human CTCF cDNAs; Figure 2A). Filippova et al teach that the encoded CTCF protein binds DNA, and finger 11 is absolutely required for binding to the P2-proximal site A of human *c-myc* (e.g., page 2807, Different combinations of CTCF Zn fingers bind to divergent sequences in the chicken and human *c-myc* promoters; Figure 7C). Finger 11 of the CTCF DNA-binding protein contains the amino acid sequence **C**SK**C**GKTFTRRNTMAR**H**ADN**C** (e.g., Figure 2A). Thus, zinc finger 11 is a non-canonical zinc finger that contains two amino acids between the two amino-terminal zinc coordinating cysteine residues, and three amino acids between the two carboxy-terminal zinc coordinating residues. The two carboxy-terminal zinc coordinating residues consist of a histidine residue that is amino terminal to a cysteine residue.

Because Barbas, III et al disclose nucleic acid molecules encoding a zinc finger protein comprising a zinc finger domain from any naturally occurring protein that has been used as a framework for modifying the DNA binding specificity according to known design rules, and Filippova et al teach a nucleic acid molecule encoding a zinc finger protein that binds DNA, it would have been obvious to one or ordinary skill in the art at the time the invention was made to include the framework sequence encoding finger 11 (i.e., the CCHC zinc finger) of the CTCF protein of Filippova et al in the nucleic acid molecules of Barbas, III et al, where the finger 11 sequence has been modified to include a recognition helix that is engineered to bind the a target

nucleic acid sequence taught by Barbas, III et al, to achieve the predictable result of making a polynucleotide that encodes a zinc finger polypeptide that binds to a plant promoter sequence containing the target nucleic acid sequence of Barbas, III et al. With respect to claims 28 and 54, which require the non-canonical zinc finger component to be the third zinc finger component or the first zinc finger component, respectively, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the zinc finger so Barbas III et al and Filippova et al in an order from N-terminus to C-terminus such that the non-canonical zinc fingers are present at the first and/or third zinc fingers. However, it is noted that the claims do not explicitly impose a linear order to the first, second and third zinc fingers.

Furthermore, one would have been motivated to include the sequence encoding the CCHC type zinc fingers of finger 11 of Filippova et al in order to expand the repertoire of available zinc finger nucleotide-binding proteins encoded by the polynucleotides. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 25-28, 30-32, 36, 39-41 and 53-57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Barbas, III et al (US Patent No. 7,329,728 B1; see the entire reference) in view of Filippova et al (Molecular and Cellular Biology, Vol. 16, No. 6, pages 2802-2813, June 1996; see the entire reference).

Barbas, III et al teach nucleic acid molecules encoding fusion proteins, expression vectors containing the nucleic acids, and cells containing the expression vectors, where the cells are plant

cells (e.g., column 2, lines 18-24; column 3, lines 12-14). Further, Barbas, III et al teach compositions comprising the nucleic acid molecule and a pharmaceutically acceptable excipient (e.g., column 5, lines 49-56). Barbas, III et al teach that the fusion protein encoded by the nucleic acid molecule includes at least one DNA binding domain, at least one ligand binding domain, and at least one transcription modulating domain, and regulates expression by binding to a target sequence in a promoter (e.g., column 2, lines 59-67; column 5, line 66 to column 6, line 22; column 9, lines 56-60; column 17, lines 41-50; paragraph bridging columns 17-18). The transcription modulating domain may be a transcription activation domain (e.g., column 29, lines 30-34). In a preferred embodiment, the DNA binding domain includes at least three zinc finger modular units and binds to at least nine nucleotides (e.g., paragraph bridging columns 2-3; column 3, lines 59-64; paragraph bridging columns 20-21). For example, six zinc fingers will bind to a sequence of 18-bp (e.g., paragraph bridging columns 20-21). Barbas, III et al teach that it is advantageous to use zinc fingers, because of the ability to construct zinc fingers with unique specificity, which permits targeting and ligand-dependent control of expression of specific endogenous genes or exogenously administered genes (e.g., column 19, lines 28-35). Barbas, III et al teach that rules for creating synthetic zinc fingers with specificity to any desired target sequence are known (e.g., column 19, lines 36-49). Barbas, III et al teach that a zinc finger-nucleotide binding peptide domain contains a unique heptamer within the alpha-helical domain of the polypeptide, which heptameric sequence determines the binding specificity to the target nucleotide (e.g., column 20, lines 43-53). Further, Barbas, III et al teach that any framework sequences known in the art to function as part of a zinc finger protein can be modified to include a peptide nucleotide-binding domain (e.g., column 20, line 43 to column 21, line 20).

Barbas, III et al do not teach the isolated polynucleotide, where the polynucleotide encodes a non-canonical zinc finger component comprising a beta turn comprising two amino-terminal zinc coordinating cysteine residues separated by two amino acids and an alpha helix comprising one carboxy-terminal zinc coordinating histidine residue and one carboxy-terminal cysteine residue, where the carboxy-terminal histidine residue is amino terminal to the carboxy-terminal cysteine residue and the histidine and cysteine residues are separated by three amino acids.

Filippova et al teach nucleic acid molecules encoding the 11 zinc fingers of CTCF protein (e.g., page 2803, Isolation of human CTCF cDNAs; Figure 2A). Filippova et al teach that the encoded CTCF protein binds DNA, and finger 11 is absolutely required for binding to the P2-proximal site A of human *c-myc* (e.g., page 2807, Different combinations of CTCF Zn fingers bind to divergent sequences in the chicken and human *c-myc* promoters; Figure 7C). Finger 11 of the CTCF DNA-binding protein contains the amino acid sequence **C**SK**C**GKTFTRRNTMAR**H**ADN**C** (e.g., Figure 2A). Thus, zinc finger 11 is a non-canonical zinc finger that contains two amino acids between the two amino-terminal zinc coordinating cysteine residues, and three amino acids between the two carboxy-terminal zinc coordinating residues. The two carboxy-terminal zinc coordinating residues consist of a histidine residue that is amino terminal to a cysteine residue.

Because Barbas, III et al disclose nucleic acid molecules encoding a zinc finger protein comprising a zinc finger domain from any naturally occurring protein that has been used as a framework for modifying the DNA binding specificity according to known design rules, and Filippova et al teach a nucleic acid molecule encoding a zinc finger protein that binds DNA, it

would have been obvious to one or ordinary skill in the art at the time the invention was made to include the framework sequence encoding finger 11 (i.e., the CCHC zinc finger) of the CTCF protein of Filippova et al in the nucleic acid molecules of Barbas, III et al, where the finger 11 sequence has been modified to include a recognition helix that is engineered to bind the a target nucleic acid sequence taught by Barbas, III et al, to achieve the predictable result of making a polynucleotide that encodes a zinc finger polypeptide that binds to a plant promoter sequence containing the target nucleic acid sequence of Barbas, III et al. With respect to claims 28 and 54, which require the non-canonical zinc finger component to be the third zinc finger component or the first zinc finger component, respectively, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the zinc finger so Barbas III et al and Filippova et al in an order from N-terminus to C-terminus such that the non-canonical zinc fingers are present at the first and/or third zinc fingers. However, it is noted that the claims do not explicitly impose a linear order to the first, second and third zinc fingers.

Furthermore, one would have been motivated to include the sequence encoding the CCHC type zinc fingers of finger 11 of Filippova et al in order to expand the repertoire of available zinc finger nucleotide-binding proteins encoded by the polynucleotides. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 37 is rejected under 35 U.S.C. 103(a) as being unpatentable over Barbas, III et al (US Patent No. 7,329,728 B1; see the entire reference) in view of Filippova et al (Molecular and

Cellular Biology, Vol. 16, No. 6, pages 2802-2813, June 1996; see the entire reference) as applied to claims 25-28, 30-32, 36, 39-41 and 53-57 above, and further in view of Guyer et al (Genetics, Vol. 149, pages 633-639, 1998, cited in a prior action; see the entire reference).

The combined teachings of Barbas, III et al and Filippova et al are described above and applied as before.

Barbas, III et al and Filippova et al do not teach the polynucleotide where the activation domain is a maize C1 activation domain.

Guyer et al teach *Arabidopsis* plants comprising a stably integrated hybrid transcription factor, and plants comprising an activatable transgene, where the hybrid transcription factor and activatable transgene are brought together in the same cell by fertilization (e.g. paragraph bridging pages 633-634). Specifically, Guyer et al teach a GAL4 DNA binding domain fused to a maize C1 transcription activation domain as the hybrid transcription factor, and a reporter transgene controlled by a synthetic promoter comprising ten GAL4 DNA binding sites (e.g. paragraph bridging pages 633-634; Figure 1). Further, Guyer et al teach that many positive transcriptional regulatory factors are modular, consisting of a DNA-binding domain and an activation domain and that fusing combinations of these elements derived from different kingdoms results in the production of diverse hybrid factors having defined DNA-binding specificity and transcriptional activation function with advantages over expression under direct control by a natural promoter (e.g. page 633, left column; page 638, paragraph bridging columns).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the polynucleotide to comprise a C1 activation domain taught by Guyer et al

because Barbas, III et al teach it is within the skill of the art to make a plant cell comprising the polynucleotide where the polynucleotide encodes a zinc finger-nucleotide binding polypeptide that activates expression of a gene operably linked to the target nucleotide sequence, and Guyer et al teach that the maize C1 activation domain functions in a plant cell to activate transcription from a heterologous DNA binding domain.

One would have been motivated to specifically use the maize C1 activation domain, because it was known in the art to function in plants. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

(10) Response to Argument

A. With respect to the rejection of claims 25-28, 30-32, 36-37, 39-41, 53-57 under 35 U.S.C. 103(a) as being unpatentable over Barbas, III et al (US Patent No. 7,151,201 B2) in view of Filippova et al (Molecular and Cellular Biology, Vol. 16, No. 6, pages 2802-2813, June 1996), Appellant's arguments filed 10/15/2009 have been fully considered but they are not persuasive.

At pages 5-11, Appellant asserts that claims 25-28, 30-32, 36-37, 39-41, 53-57 are not obvious over Barbas, III et al in view of Filippova et al for the following reasons: (a) the references do not teach or suggest each element of the rejected claims; (b) the cited art teaches away from the claimed invention; and (c) it would not have been predictable to modify the recognition helix of the CCHC zinc finger of Filippova et al to bind to a gene in a plant cell. Each of these reasons will be addressed in turn.

(a) Assertion that the references do not teach or suggest each element of the rejected claims

At page 6, paragraph 1, Appellant states that "the combination of references must disclose a polynucleotide that encodes a non-naturally occurring zinc finger protein that includes a non-canonical (non-Cys2His2) zinc finger with the recited number of amino acids between zinc coordinating residues and in which the recognition helix of the non-canonical zinc finger has been altered to bind to a target site in a plant gene."

Independent claim 30 is drawn to an isolated polynucleotide encoding a non-naturally-occurring zinc-finger binding protein comprising a non-canonical zinc finger component, wherein: (i) said non-canonical zinc finger component contains a beta turn comprising two amino-terminal zinc coordinating cysteine or histidine residues and an alpha helix comprising two carboxy-terminal zinc coordinating cysteine or histidine residues, wherein at least one of the zinc coordinating residues is a histidine residue and at least one of the zinc coordinating residues is a cysteine residue; (ii) the non-canonical zinc finger component comprises 2, 3 or 4 amino acids between the two amino-terminal zinc coordinating residues, and 1, 2, 3, 4, 6 or 7 amino acids between the two carboxy-terminal zinc coordinating residues; and (iii) the non-canonical zinc-finger binding domain protein comprises a recognition helix of at least 7 amino acids in length, therein the recognition helix is non-naturally occurring and is engineered to bind to a target nucleic acid sequence in a plant cell. Independent claim 56 is drawn to an isolated polynucleotide encoding a non-naturally occurring zinc-finger binding protein comprising a non-canonical zinc finger component, wherein: (i) said non-canonical zinc finger component contains a beta turn comprising two amino-terminal zinc coordinating cysteine and an alpha helix

comprising two carboxy- terminal zinc coordinating cysteine or histidine residues, wherein one of the carboxy- terminal zinc coordinating residues is a histidine residue and one of the carboxy-terminal zinc coordinating residues is a cysteine residue; (ii) the non-canonical zinc finger component comprises 2 amino acids between the two amino-terminal zinc coordinating cysteine residues; and (iii) the protein comprises a non-naturally occurring recognition helix that is engineered to bind to a target nucleic acid sequence.

All of the structural and functional limitations of the claims are met by the combined teachings of Barbas, III et al and Filippova et al. Barbas, III et al teach polynucleotides encoding non-naturally occurring zinc finger proteins in which the recognition helix of each of the zinc finger components is engineered to bind to a promoter of a gene in a plant cell (e.g., column 3, lines 13-50; column 10, lines 55-67; column 11, lines 14-35; column 18, lines 48-64; column 19, lines 28-34 and 53-57; Table 2 at columns 56-57). Filippova et al teach a polynucleotide encoding a zinc finger protein comprising a non-canonical zinc finger of the sequence **C**SK**C**GKTFTRRNTMAR**H**ADN**C** (e.g., Figure 2A). The non-canonical zinc finger of Filippova et al meets all the structural limitations of the claims with regard to the non-canonical zinc finger component, except that it does not comprise a recognition helix engineered to bind to a target nucleic acid sequence in a plant cell; however, Barbas, III et al teach it is within the skill of the art to use any naturally occurring zinc finger protein as a framework in a zinc finger protein engineered to bind to a target sequence in the promoter of a gene in a plant cell (e.g., column 21, lines 8-15; column 23, lines 8-11). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include the framework sequence encoding finger 11 of the CTCF protein of Filippova et al in the polynucleotide of Barbas, III et

al, where the finger 11 sequence has been modified to include a recognition helix that is engineered to bind the target nucleic acid sequence taught by Barbas, III et al, to achieve the predictable result of making a polynucleotide that encodes a zinc finger polypeptide that binds to a plant promoter sequence.

At page 6, paragraph 2, Appellant notes that the rejection appears to be based on the assertion that the teachings in Barbas, III et al ('201) regarding altering the recognition helices of any naturally occurring zinc finger within the context of its naturally occurring framework can be applied to Filippova's CTCF protein to result in a molecule as claimed.

Appellant's arguments suggest that Barbas, III et al ('201) teach that one must use the naturally occurring framework as a whole without any modification. A fair reading of Barbas, III et al indicates that the use of the zinc finger framework is highly flexible in the design of zinc finger proteins to achieve the goal of providing a zinc finger protein to bind any target nucleotide sequence of interest. It is noted that Barbas, III et al teach that "'zinc finger protein, (zinc finger polypeptide, or ZFP)' refers to a polypeptide having nucleic acid, e.g., DNA, binding domains that are stabilized by zinc" (column 10, lines 55-57). Barbas, III et al teach that the zinc finger protein is composed of individual DNA binding domains that are typically referred to as "fingers," and each finger typically binds from two to four base pairs of DNA, typically three or four base pairs of DNA (e.g., column 10, lines 57-64). Barbas, III et al teach that each zinc finger usually comprises approximately 30 amino acids, zinc-chelating residues, and a DNA-binding subdomain (e.g., column 10, lines 65-67). Barbas, III et al define "framework" to mean "that the protein or peptide sequence within the naturally occurring zinc finger protein that is involved in non-sequence specific binding with a target nucleotide sequence is not substantially

changed from its natural sequence" (column 11, lines 15-19). Barbas, III et al teach that ZIF268 is a useful framework, however, others are suitable (e.g., column 21, lines 8-10). Barbas, III et al teach that the Zif268 framework may be truncated, expanded, and/or mutagenized in order to change the function of a nucleotide sequence encoding a zinc finger nucleotide binding motif; however, other zinc finger nucleotide binding proteins are known to those of skill in the art and can be used (e.g., column 21, lines 10-15; column 22, line 51 to column 23, line 11). In other words, the framework protein can be truncated to less than its full complement of zinc fingers. Further, different zinc finger domains may be combined to create new combinations of zinc fingers and zinc finger protein sequences (e.g., paragraph bridging columns 19-20; column 20, lines 30-40; column 21, lines 8-15). According to the teachings of Barbas, III et al it would have been within the skill of the art to use a zinc finger framework that includes a single zinc finger from any known protein, to mutagenize the zinc finger domain within the recognition helix, and to combine that zinc finger domain with other zinc finger domains (e.g., column 1, line 53 to column 2, line 6; column 3, lines 36-43; column 4, line 59 to column 5, line 11; column 18, line 47 to column 20, line 40).

At page 6, paragraph 3, Appellant states, "It is undisputed that Barbas '201 teaches only that the recognition helices can be altered in the context of the naturally occurring protein as a whole." Appellant cites column 11, lines 14-19; column 20, lines 3-10; column 21, lines 8 to 20; column 22, lines 51-60; column 22, lines 61 to column 23, line 61; column 23, lines 8-11; column 23, lines 12-15; and column 23, lines 23-25.

This argument is not found persuasive. For the reasons set forth above, Barbas, III et al teach polynucleotides encoding a zinc finger protein that comprises less than the whole, naturally

occurring zinc finger protein framework. Furthermore, the complete passage at column 11, lines 14-35 states the following:

> As used herein, "framework (or backbone) derived from a naturally occurring zinc finger protein" means that the protein or peptide sequence within the naturally occurring zinc finger protein that is involved in non-sequence specific binding with a target nucleotide sequence is not substantially changed from its natural sequence. For example, such framework (or backbone) derived from the naturally occurring zinc finger protein maintains at least 50%, and preferably, 60%, 70%, 80%, 90%, 95%, 99% or 100% identity compared to its natural sequence in the non-sequence specific binding region. Alternatively, the nucleic acid encoding such framework (or backbone) derived from the naturally occurring zinc finger protein can be hybridizable with the nucleic acid encoding the naturally occurring zinc finger protein, either entirely or within the non-sequence specific binding region, under low, medium or high stringency condition. Preferably, the nucleic acid encoding such framework (or backbone) derived from the naturally occurring zinc finger protein is hybridizable with the nucleic acid encoding the naturally occurring zinc finger protein, either entirely or within the non-sequence specific binding region, under high stringency condition.

Although Barbas, III et al initially state that the framework is "not substantially changed," the further description provided by Barbas, III et al allows for a significant amount of change, where the sequence is 50% identical or hybridizable with the nucleic acid encoding the naturally occurring zinc finger protein under low, medium or high stringency. Furthermore, the passage cited as column 21, lines 8-20 indicates that zinc finger frameworks can be <u>truncated</u>, expanded, and/or mutagenized. Contrary to Appellant's assertion, the teachings of Barbas, III et al are <u>not</u> limited to the alteration of a recognition helix in the context of the naturally occurring protein as a whole. Furthermore, Barbas, III et al teach that zinc finger proteins or polypeptides are composed of at least one finger, and zinc finger proteins can be linked via a linker to at least a second DNA binding domain, which optionally is a second zinc finger polypeptide, where the zinc finger polypeptides are engineered to recognize a selected target site in the gene of choice

(e.g., column 10, lines 55-62; column 19, line 64 to column 20, line 40). According to the teachings of Barbas, III et al it would have been within the skill of the art to use a zinc finger framework that includes a single zinc finger from any known protein, to mutagenize the zinc finger domain within the recognition helix, and to combine that zinc finger domain with other zinc finger domains (e.g., column 1, line 53 to column 2, line 6; column 3, lines 36-43; column 4, line 59 to column 5, line 11; column 18, line 47 to column 20, line 40).

At page 8, paragraph 1, Appellant states, "the issue is whether the references (or state of the art as a whole) teach that recognition helix within any framework can be altered as described in Barbas '201 and the resulting protein would necessarily bind to a target site in a plant gene, as claimed." Appellant asserts that when using Filippova's CTCF as the framework (backbone), the skilled artisan must have been taught by Barbas '201 that altering the recognition helix of the only C3H finger (the 11th finger) of CTCF would result in a protein in which this altered C3H finger bound to a target site in a plant gene. At page 8, paragraph 2, Appellant states that such teachings are completely lacking from Barbas '201, Filippova and the state of the art, and the only frameworks that Barbas actually shows are functional are canonical Cys2His2 proteins, particularly Zif268 and/or TFIIIA. At page 9, paragraph 2, Appellant states that Barbas '201 and Filippova fail to teach or suggest the claimed elements (e.g., a protein comprising a non-canonical finger with a recognition helix that is engineered to bind to a target site in a plant gene).

These arguments are not found persuasive. As discussed above, Barbas, III et al suggest polynucleotides encoding zinc finger polypeptides designed to bind to a particular sequence in a plant gene, where the zinc finger polypeptide contains a zinc finger framework from a truncated

zinc finger protein. Filippova et al also teach that it is within the skill of the art to engineer truncated versions of the naturally occurring zinc finger protein CTCF (e.g., page 2803, left column, 2nd full paragraph; page 2807, right column, 2nd full paragraph). Filippova et al use pairs of PCR primers to amplify different combinations of zinc finger domains within the CTCF protein (e.g., page 2803, left column, 2nd full paragraph). Barbas, III et al teach that the construction of a polynucleotide encoding the zinc finger polypeptide can be performed using primers for PCR amplification of zinc finger domains to create new combinations of fingers and ZFP sequences (e.g., column 20, lines 15-40). Barbas, III et al teach that the recognition helix can be changed so that the zinc finger protein is designed to bind any desired target sequence in a plant gene (e.g., paragraph bridging columns 1-2; column 3, lines 23-43; column 19, lines 28-34; paragraph bridging columns 30-31). Contrary to Appellant's assertion, the rejection of record is not based upon the sole modification of the recognition helix of the 11th finger in the context of the whole, naturally occurring CTCF protein, such that the sole modification of finger 11 would result in a zinc finger protein that binds to a target sequence in the promoter of a plant gene. Rather, it would have been obvious to one or ordinary skill in the art at the time the invention was made to include the framework sequence encoding finger 11 (i.e., the CCHC zinc finger) of the CTCF protein of Filippova et al in the nucleic acid molecules of Barbas, III et al, where the finger 11 sequence has been modified to include a recognition helix that is engineered to bind the a target nucleic acid sequence taught by Barbas, III et al, to achieve the predictable result of making a polynucleotide that encodes a zinc finger polypeptide that binds to a plant promoter sequence containing the target nucleic acid sequence of Barbas, III et al. It would have been within the skill of the art to use a fragment of the CTCF protein, including finger 11, as a

framework for the construction of a zinc finger protein as taught by Barbas, III et al, where the recognition helix of each of the zinc fingers is engineered to bind to a target nucleotide sequence of interest in a plant gene (e.g., paragraph bridging columns 1-2; column 3, lines 34-43; column 11, lines 14-24; column 19, lines 53-57).

With regard to the recognition helices to be used to create the binding specificity of the engineered zinc finger protein encoded by the polynucleotide, Barbas, III et al specifically teach the use of the binding domain/nucleotide binding partner pairs disclosed in U.S. Pat. No. 5,789,538, WO96/06166, and WO 00/23464 (column 19, lines 53-57). Each of these references has been made of record in the instant application. U.S. Patent No. 5,789,538 was cited as reference A26 on the IDS filed 5/11/2005. WO 96/06166 was cited as reference B5 on the IDS filed 5/11/2005. WO 00/23464 was cited as reference B-5 on the IDS filed 4/15/2003. WO 00/23464 teaches the amino acid residues within the alpha-helical domain of zinc fingers that specifically bind to target nucleotide sequences of the formula 5'-GNN-3', where N is A, C, G, or T (e.g., page 1, lines 10-15; page 5, lines 20-30). WO 00/23464 teaches that nucleic acid recognition is achieved through specific amino acid side chain contacts originating from the alpha-helix of the zinc finger domain, which typically binds 3 bases pairs of DNA sequence (e.g., page 4, lines 29-31). The specific alpha-helical regions and target nucleic acid sequences are disclosed in Table 1 at page 13. Further, WO 00/23464 teaches that simple covalent linkage of multiple zinc finger domains allows the recognition of extended asymmetric sequences of DNA (e.g., page 4, lines 34-36). Accordingly, the teachings of Barbs, III et al ('201) with regard to engineering a zinc finger protein to bind to a target sequence of a plant promoter, where the

target sequence is composed of contiguous GNN triplets is supported by the teachings of the prior art.

At page 8, 1st full paragraph, Appellant asserts that "the skilled artisan must have been taught by Barbas '201 that altering the recognition helix of the only C3H finger (the 11th finger) of CTCF would result in a protein in which this altered C3H finger bound to a target site in a plant gene" (page 8, 1st full paragraph). If Barbas, III et al specifically taught the modification of the 11th finger of CTCF (the CCHC zinc finger), then the rejection would have been made under 35 U.S.C. 102. A rejection based upon the combined teachings of Barbas '201 and Filippova et al is proper even though the invention is not identically disclosed or described in Barbas '201 alone, because the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art.

Barbas, III et al teach that any naturally occurring zinc finger protein can be used as a framework (or backbone) to derive a non-naturally occurring zinc finger with DNA binding specificity determined by alterations in the alpha helix of the zinc finger by using known design rules (e.g., column 10, lines 55-67; column 11, lines 14-35; column 19, lines 28-34 and 57; column 21, lines 8-39; column 22, line 51 to column 25, line 9). Barbas, III et al teach the use of any zinc finger and define the term "zinc finger" to mean "a polypeptide having nucleic acid, e.g., DNA binding domains that are stabilized by zinc" (e.g., column 10, lines 55-57; column 18, lines 47-49). The zinc finger of Filippova et al meets the definition of "zinc finger" provided by Barbas, III et al. Because Barbas, III et al disclose nucleic acid molecules encoding a zinc finger protein comprising a zinc finger domain from any naturally occurring protein that has been used

as a framework for modifying the DNA binding specificity according to known design rules, and Filippova et al teach a nucleic acid molecule encoding a zinc finger protein that binds DNA, it would have been obvious to one or ordinary skill in the art at the time the invention was made to include the framework sequence encoding finger 11 (i.e., the CCHC zinc finger) of the CTCF protein of Filippova et al in the nucleic acid molecules of Barbas, III et al, where the finger 11 sequence has been modified to include a recognition helix that is engineered to bind the a target nucleic acid sequence taught by Barbas, III et al, to achieve the predictable result of making a polynucleotide that encodes a zinc finger polypeptide that binds to a plant promoter sequence containing the target nucleic acid sequence of Barbas, III et al.

(b) Assertion that the cited art teaches away from the claimed invention

At page 8, paragraph 2, Appellant asserts that Filippova teaches away from using CTCF as a framework because of the fact that this 11-finger framework (backbone) uses different finger combinations to bind to a variety of different target sites. Thus, Appellant asserts that altering the recognition helix of finger 11 of CTCF has no relevance to DNA binding of the protein, inasmuch as the only non-canonical finger (finger 11) is not necessarily involved in DNA binding.

These arguments are not found persuasive. Filippova et al teach that finger 11 is involved in specifically binding to the P2-proximal site A of the human *c-myc* gene (e.g., page 2807, right column, 2^{nd} full paragraph; Figure 7). Filippova et al do not criticize, discredit, or otherwise discourage the use of CTCF zinc finger domains as frameworks in engineered zinc finger proteins. As discussed above, Barbas, III et al suggest polynucleotides encoding zinc

finger polypeptides designed to bind to a particular sequence in a plant gene, where the zinc finger polypeptide contains a zinc finger framework from a truncated zinc finger protein. Barbas, III et al teach the use of any zinc finger and define the term "zinc finger" to mean "a polypeptide having nucleic acid, e.g., DNA binding domains that are stabilized by zinc" (e.g., column 10, lines 55-57; column 18, lines 47-49). The zinc finger of Filippova et al meets the definition of "zinc finger" provided by Barbas, III et al. Filippova et al also teach that it is within the skill of the art to engineer truncated versions of the naturally occurring zinc finger protein CTCF (e.g., page 2803, left column, 2nd full paragraph; page 2807, right column, 2nd full paragraph). Filippova et al use pairs of PCR primers to amplify different combinations of zinc finger domains within the CTCF protein (e.g., page 2803, left column, 2nd full paragraph). Barbas, III et al teach that the construction of a polynucleotide encoding the zinc finger polypeptide can be performed using primers for PCR amplification of zinc finger domains to create new combinations of fingers and ZFP sequences (e.g., column 20, lines 15-40). Filippova et al teach fragments of the CTCF protein that predictably bind a target DNA sequence through the use of finger 11 (e.g., page 2807, right column, 2nd full paragraph; Figure 7). For example, fingers 3-11 bind site A of the *c-myc* gene (e.g., Figure 7F). Removal of finger 11 from the polypeptide abolishes binding to site A (e.g., Figure 7C). Finger 11 is absolutely required for binding to site A. Barbas, III et al teach that the recognition helix of any zinc finger can be changed so that the zinc finger protein is designed to bind any desired target sequence in a plant gene (e.g., paragraph bridging columns 1-2; column 3, lines 23-43; column 19, lines 28-34; paragraph bridging columns 30-31). Even though the full-length, naturally occurring CTCF protein uses different combinations of zinc fingers to bind target sites, finger 11 is known to bind

to a target DNA sequence, and it would have been within the skill of the art to use CTCF zinc finger domain(s) as a framework to engineer zinc finger proteins that bind to contiguous sequences according to the teachings of Barbas, III et al.

(c) Assertion that it would not have been predictable to modify the recognition helix of the CCHC zinc finger of Filippova et al to bind to a gene in a plant cell

At page 5, paragraph 3, Appellant asserts that Barbas '201 and Filippova do not establish that it was predictable to modify the recognition helix region of C3H zinc fingers having the claimed structure to bind to a gene in a plant cell. At pages 9-11, Appellant asserts that there is no combination of Barbas '201 and Filippova that establishes that the proteins encoded by the claimed polynucleotides were a predictable use of allegedly known elements. Appellant notes that Barbas, III et al do not teach non-canonical zinc finger domains set forth in claims 30 and claims dependent therefrom, namely zinc fingers having 2-4 amino acid residues between the amino terminal zinc coordinating residues and 1, 2, 3, 4, 6, or 7 amino acid residues between the carboxy terminal zinc coordinating residues. Thus, Appellant asserts that Barbas '201 fails to teach using C3H backbones of the claimed structure and does not teach that it was predictable to modify the backbones and/or recognition helixes of Filippova's CTCF protein, including the lone C3H zinc finger, to arrive at the claimed plant gene-binding proteins. Further, Appellant notes that finger 11 does not always participate in binding to DNA in the context of the naturally occurring protein and asserts that binding will remain unpredictable. Appellant asserts that the protein may bind to a site using only fingers 2-7.

These arguments are not found persuasive. The rejection of record is not based upon a polynucleotide encoding the entire naturally occurring CTCF protein as a backbone for the design of a zinc finger polypeptide. Filippova et al teach fragments of the CTCF protein that predictably bind a target DNA sequence through the use of finger 11 (e.g., page 2807, right column, 2nd full paragraph; Figure 7). For example, fingers 3-11 bind site A of the *c-myc* gene (e.g., Figure 7F). Removal of finger 11 from the polypeptide abolishes binding to site A (e.g., Figure 7C). Finger 11 is absolutely required for binding to site A. Because finger 11 of CTCF is known to be a DNA-binding zinc finger, it would have been predictable to make a nucleic acid sequence encoding the zinc finger in a polynucleotide taught by Barbas, III et al. The essential function of the encoded zinc finger is retained (i.e., ability to bind DNA); however, the specificity of DNA binding will be altered according to the rules taught by Barbas, III et al, such that the polynucleotide encodes an engineered zinc finger protein that binds to a target sequence in a plant gene promoter.

Appellant asserts that additional publications by Barbas evidence that the skilled artisan believed that framework residues could impact binding and, as such, only when the recognition helix of certain Cys2His2 frameworks were altered was binding function in any way predictable. Appellant points to Barbas '728 as teaching that altered recognition helices function only in certain frameworks (Barbas '728 col. 42, lines 19-24):

> The framework residues play a role in affinity and specificity. Thus, amino acid positions -2 to 6 of the DNA recognition helices are either grafted into a Zif268 (Pavletich et al. (1991) Science 252:809-817) or an SplC framework (Desjarlais et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:2256-2260).

This argument is not found persuasive. Barbas, III et al (US Patent No. 7,329,728) teach the following with regard to zinc finger-nucleotide binding peptide domains (column 20, lines 43-53, emphasis added):

> A zinc finger-nucleotide binding peptide domain contains a unique heptamer (contiguous sequence of 7 amino acid residues) within the α-helical domain of the polypeptide, **which heptameric sequence determines binding specificity** to a target nucleotide. The heptameric sequence can be located anywhere within the α-helical domain but it is preferred that the heptamer extend from position -1 to position 6 as the residues are conventionally numbered in the art. **A peptide nucleotide-binding domain can include any β-sheet and framework sequences known in the art to function as part of a zinc finger protein**.

Finger 11 of the CTCF protein of Filippova et al is a zinc finger domain known in the art to function as part of a zinc finger protein and is shown by Filippova et al to be capable of binding to a DNA target sequence. Barbas, III et al tested fused finger 2 variants and helix grafted variants of Zif268 or Sp1C for the ability to bind their specific target sequences. All attempts to create zinc finger protein that bind to a specific target sequence were successful (e.g., column 44, line 53 to column 45, line 67; Table 4). Barbas, III et al may have selected Zif268, because it is the most well characterized of the zinc finger proteins (e.g., column 21, lines 34-40) and may have selected Sp1C due to its enhanced stability towards chelating agents (e.g., column 45, lines 21-22). Barbas, III et al do not provide evidence that other frameworks and/or combinations of zinc finger domains would not have predictably bound their target sequences. Although affinity and specificity may vary, binding to target sequences was predictable when the different frameworks were used (e.g., Table 4).

The position of Barbas, III ('728) et al is that the heptameric sequence within the α-helical domain of the zinc finger is what determines binding specificity, and any β-sheet and framework

sequences known in the art to function as part of a zinc finger protein can be predictably used (e.g., column 20, lines 43-5). This position is consistent with Appellant's remarks provided in the reply filed 9/10/2008 (paragraph bridging pages 8-9):

> Moreover, the assertion that binding functionality is unpredictable, or in any way makes the genus encompassed by the claims unduly large, is completely unsupported by the evidence of record. There is detailed disclosure in the specification indicating that it was predictable at the time of filing that binding specificity was dependent on the recognition helix sequence, <u>not</u> the zinc coordinating residues. Variability in the recognition helix region of these proteins does not affect the description of the claimed proteins - any recognition helix can be used, so long as the zinc coordinating residues of at least one finger fall into the limited genus recited in the claims. As it is admitted that cysteine and histidine both act as zinc coordinating residues, it is clear that the skilled artisan would immediately envisage any of the claimed non-canonical finger structures with the selected recognition helix to bind to the selected nucleic acid target site.

Accordingly, the combination of Barbas, III et al ('201) and Filippova et al would have yielded nothing more than predictable results to one of ordinary skill in the art at the time of the invention.

B. With respect to the rejection of claims 25-28, 30-32, 36, 39-41 and 53-57 under 35 U.S.C. 103(a) as being unpatentable over Barbas, III et al (US Patent No. 7,329,728 B1) in view of Filippova et al (Molecular and Cellular Biology, Vol. 16, No. 6, pages 2802-2813, June 1996), Appellant's arguments filed 10/15/2009 have been fully considered but they are not persuasive.

At page 11, Appellant asserts that there is no combination of Barbas '728 and Filippova that teaches or suggests the claimed molecules. Appellant states that Barbas '728 clearly teaches that not any framework zinc finger backbone can be used with assurances that the recognition helix will bind to the predicted target site (Barbas '728, column 42, lines 19-24; col. 45, lines 15-21):

> The framework residues play a role in affinity and specificity. Thus, amino acid positions -2 to 6 of the DNA recognition helices are either grafted into a Zif268 (Pavletich et al. (1991) Science 252:809-817) or an SplC framework (Desjarlais et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:2256-2260).
>
> The framework residues may play a role in affinity and specificity. For helix grafting, amino acid positions -2 to 6 of the DNA recognition helices were either grafted into a Zif268 (Pavletich et al. (1991) Science 252:809-817) or an SplC framework (Desjarlais et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:2256-2260).

At page 12, Appellant asserts that Barbas '728 teaches away from using Filippova's CTCF framework by clearly teaching that it is entirely unpredictable to alter the recognition helix of frameworks other than canonical Zif268 or Sp1 a protein of known binding specificity.

These arguments are not found persuasive. Barbas, III et al (US Patent No. 7,329,728) teach the following with regard to zinc finger-nucleotide binding peptide domains (column 20, lines 43-53, emphasis added):

> A zinc finger-nucleotide binding peptide domain contains a unique heptamer (contiguous sequence of 7 amino acid residues) within the α-helical domain of the polypeptide, **which heptameric sequence determines binding specificity** to a target nucleotide. The heptameric sequence can be located anywhere within the α-helical domain but it is preferred that the heptamer extend from position -1 to position 6 as the residues are conventionally numbered in the art. **A peptide nucleotide-binding domain can include any β-sheet and framework sequences known in the art to function as part of a zinc finger protein**.

Finger 11 of the CTCF protein of Filippova et al is a zinc finger domain known in the art to function as part of a zinc finger protein and is shown by Filippova et al to be capable of binding to a DNA target sequence. Barbas, III et al suggest the modification of any known zinc finger sequence. Barbas, III et al tested fused finger 2 variants and helix grafted variants of Zif268 or Sp1C for the ability to bind their specific target sequences. All attempts to create zinc finger protein that bind to a specific target sequence were successful (e.g., column 44, line 53 to column

45, line 67; Table 4). Barbas, III et al may have selected Zif268, because it is the most well characterized of the zinc finger proteins (e.g., column 21, lines 34-40) and may have selected Sp1C due to its enhanced stability towards chelating agents (e.g., column 45, lines 21-22). Sp1C is a completely designed consensus protein (not the naturally occurring Sp1 to which Appellant refers), and the specificity of Sp1C is determined by the helices that are grafted (e.g., column 45, lines 12-22). See Desjarlais et al PNAS, Vol. 90, pages 2256-2260, 1993 (cited as reference C38 on the IDS filed 11/15/2005). Barbas, III et al do not provide evidence that other frameworks and/or combinations of zinc finger domains would not have predictably bound their target sequences. Although affinity and specificity may vary, binding to target sequences was predictable when the different frameworks were used (e.g., Table 4). Accordingly, the combination of Barbas, III et al ('728) and Filippova et al would have yielded nothing more than predictable results to one of ordinary skill in the art at the time of the invention.

C. With respect to the rejection of claim 37 under 35 U.S.C. 103(a) as being unpatentable over Barbas, III et al (US Patent No. 7,329,728 B1) in view of Filippova et al (Molecular and Cellular Biology, Vol. 16, No. 6, pages 2802-2813, June 1996), and further in view of Guyer et al (Genetics, Vol. 149, pages 633-639, 1998), Appellant's arguments filed 10/15/2009 have been fully considered but they are not persuasive.

Appellant asserts that Guyer et al fail to cure the deficiencies of Barbas '728 and Filippova et al.

This argument is not found persuasive for the reasons set forth above with regard to the rejection of claims 25-28, 30-32, 36, 39-41 and 53-57 under 35 U.S.C. 103(a) as being

unpatentable over Barbas, III et al (US Patent No. 7,329,728 B1) in view of Filippova et al (Molecular and Cellular Biology, Vol. 16, No. 6, pages 2802-2813, June 1996).

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

/Jennifer Dunston/
Examiner, Art Unit 1636

Conferees:
/ Christopher S. F. Low /
Supervisory Patent Examiner, Art Unit 1636

/GARY BENZION/
Supervisory Patent Examiner, Art Unit 1637